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Title:	Slides for FNLCR collaborators
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Intended for:	Figures for collaborators to potentially use in writing extension for funding.
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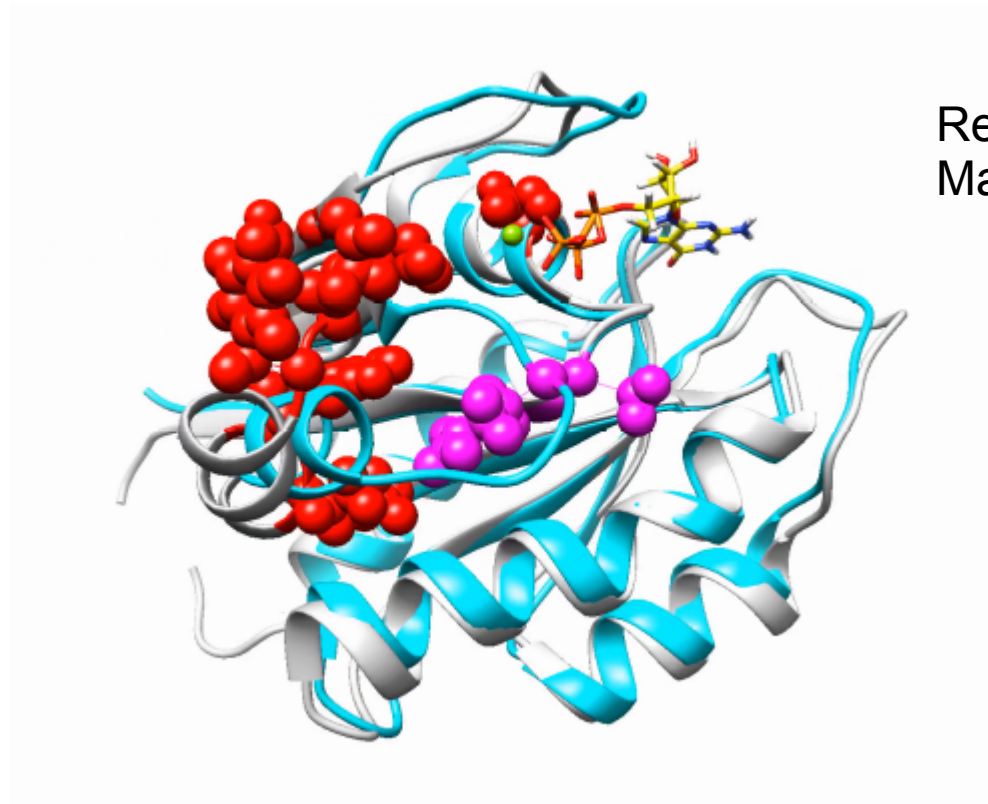
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Slides for FNLCR collaborators

Recent work, work in progress, immediately planned work

- Have begun looking into applicable crystal structures for binding partners
 - Interested in how RIT1 binds
 - How is this related to membrane binding
 - How is this related to NS mutations?
- RIT1 deactivation occurs with same kinetics that Ras does
- C terminal peptide binds preferentially to a membrane containing charged lipids
- G domain + C terminal peptide also binds to a membrane containing charged lipids

Common NS mutations



Most occur in the SWII region, and may effect membrane orientation, or may alter effector binding

Binding partners

RIT1 interacts with:

Note : those without citations are uniprot annotated but not found in literature

PAK1 (2HY8) – p21-activated kinase 1 ¹

CDC42 (20DB) – RHO GTPase involved in regulation of actin dynamics ^{1 *}

RAC1 (2RMK) – RHO GTPase involved in regulation of actin dynamics ^{1 *}

RAFRBD (3KUD) – binding determined by Y2H system. S35N mutation abrogates binding ²

P110 catalytic subunit of P13K

RALGDS-RID (1LFD) – binding by Y2H. S35N mutation abrogates binding. Prefers GTP-bound²

RLF-RID - binding determined by Y2H. S35N mutation abrogates binding. Prefers GTP-bound²

RLF (BD: 1RLF, whole: 4JGW)– binding determined by co-IP ²

AF6 - binding determined by Y2H. S35N mutation abrogates binding. Prefers GTP-bound²

PCCE1 – can find no evidence of this study in literature

PIK3CA – demonstrated via pull down assay ¹

RGL3 – Ral-GDS-like protein binds with GTP-bound Rit in an effector loop-dependent manner **likely GEF for Rit1**. Determined by yeast-2-hybrid, binds via C terminal binding domain ⁴

Par6 (1NF3) – Binding is GTP-independent Par3/6/aPKC polarity complex may contain a scaffolded RitGEF. ⁵

B-Raf (1UWH)- determined by pull down and immunoblot⁷

C-Raf (4G0N)- determined by pull down and immunoblot⁷

p38 MAPK (5MTX) – determined by immunoprecipitation ⁶

MK2 (inhibitor bound: 3R2B) – determined by immunoprecipitation ⁶

HSP27 – determined by immunoprecipitation ⁶

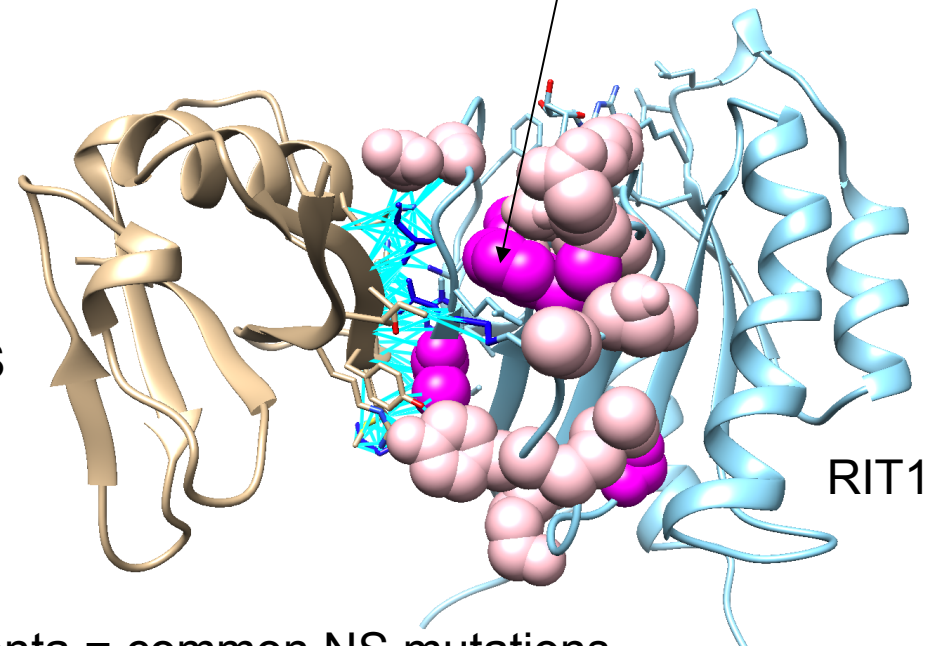
Akt (with inhibitor: 3MV5) – determined by immunoprecipitation ⁶

PIP lipids – determined by binding assay ⁸

*interaction is independent of nucleotide bound state

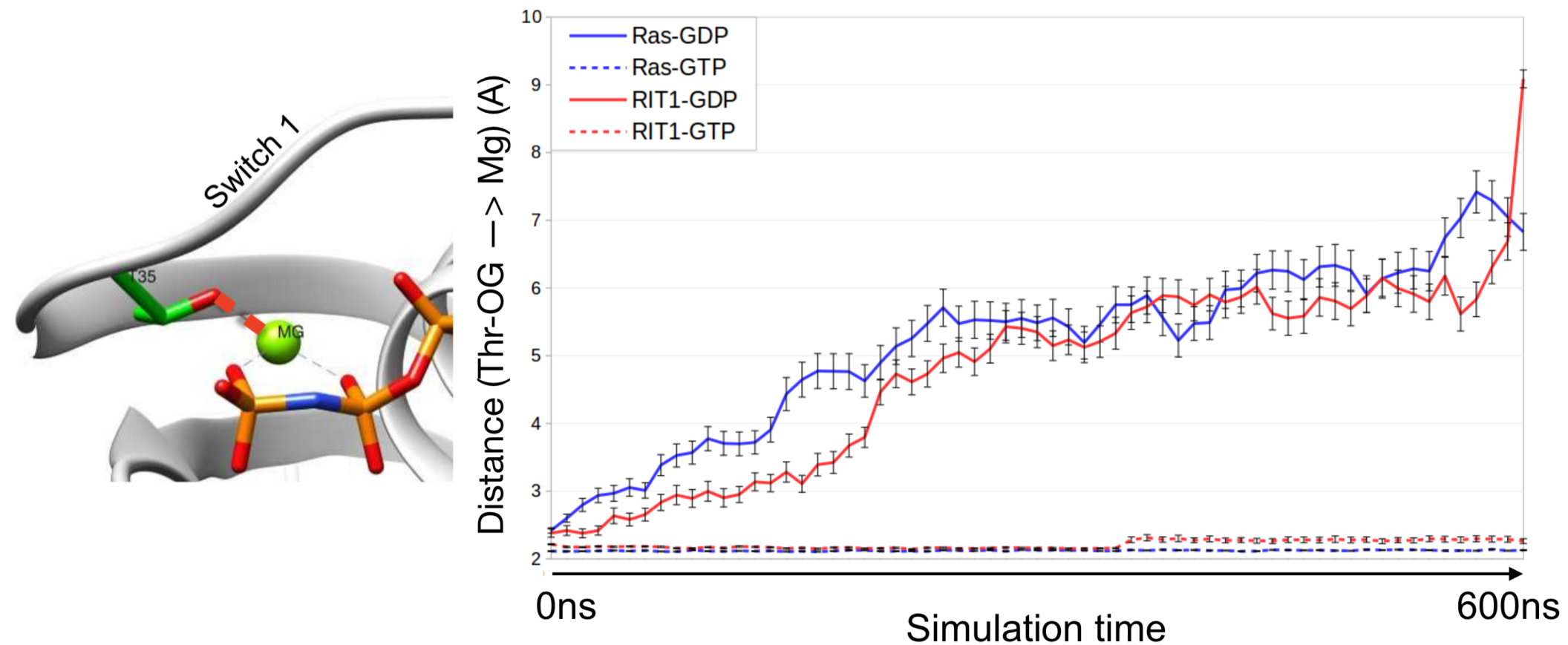
S35N abrogates binding

RALGDS
PDB: 1LFD (RALGDS bound with Ras)

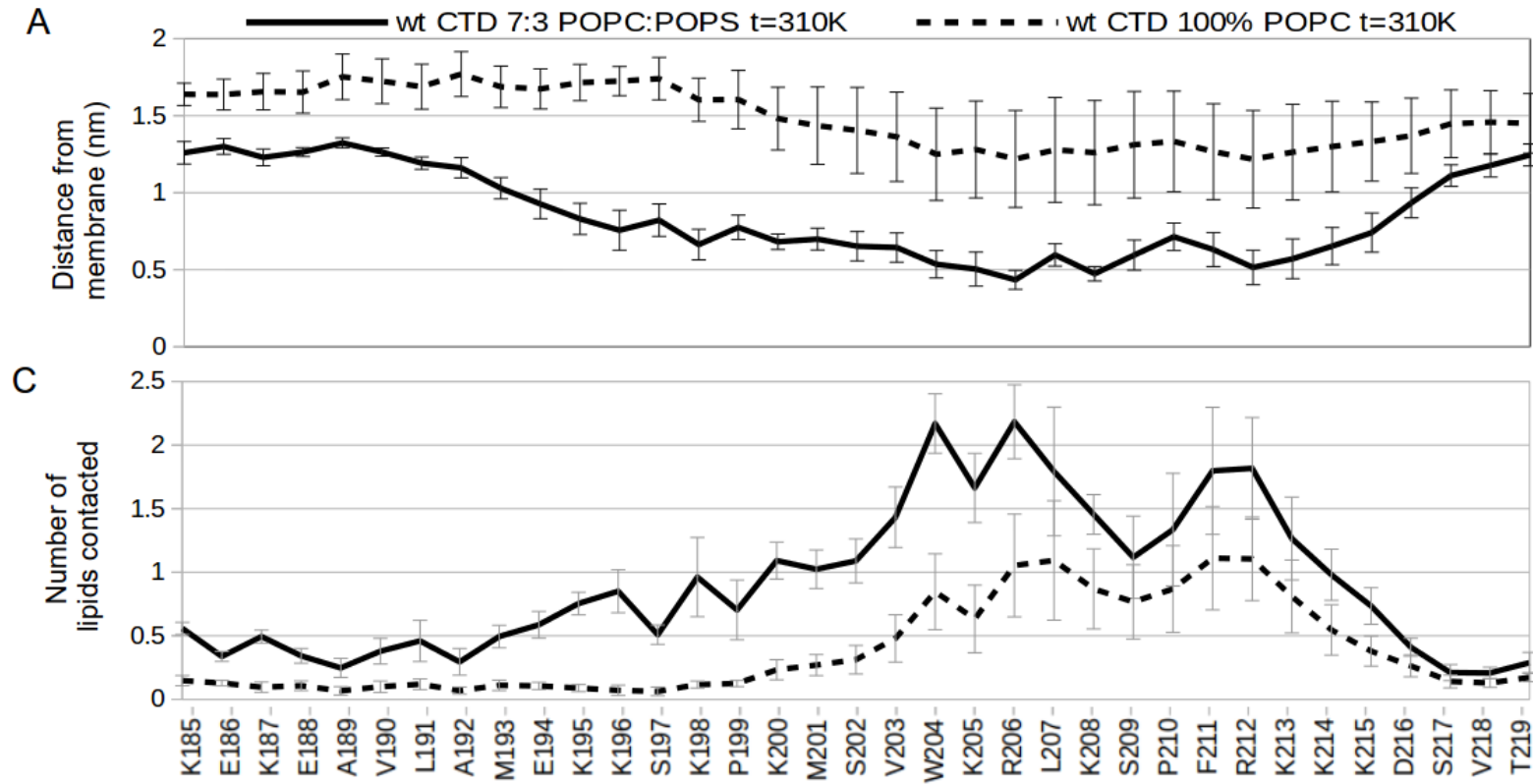
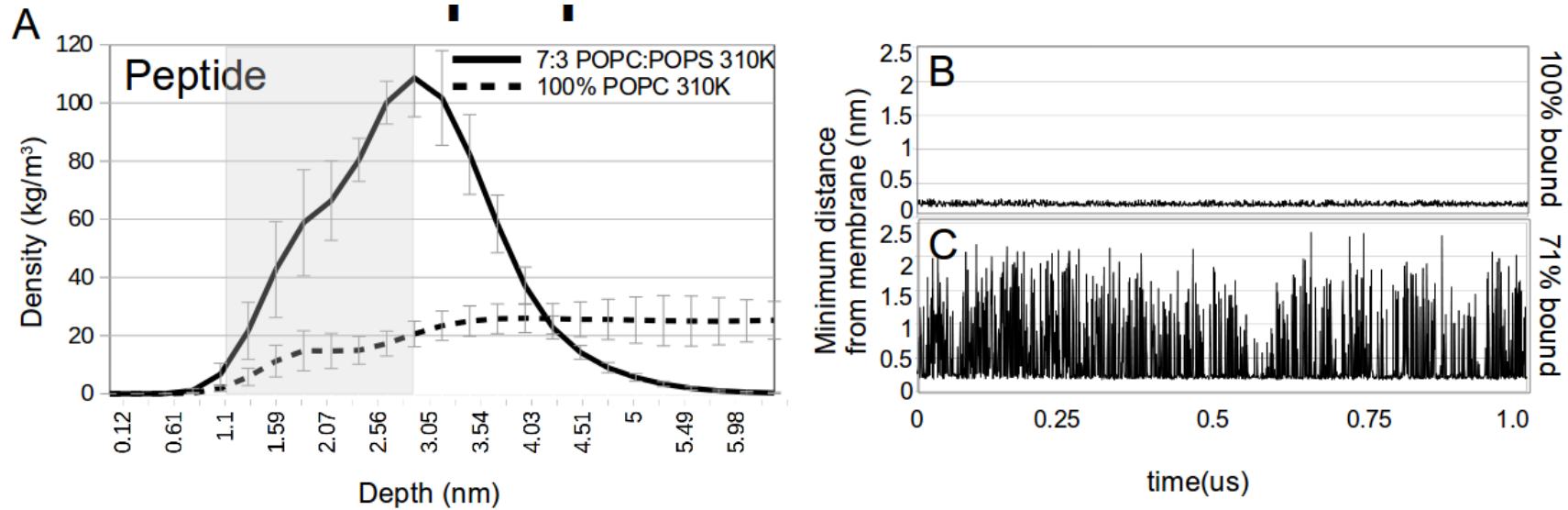


Magenta = common NS mutations
Pink = less common NS mutations
Cyan = interactions found in this alignment

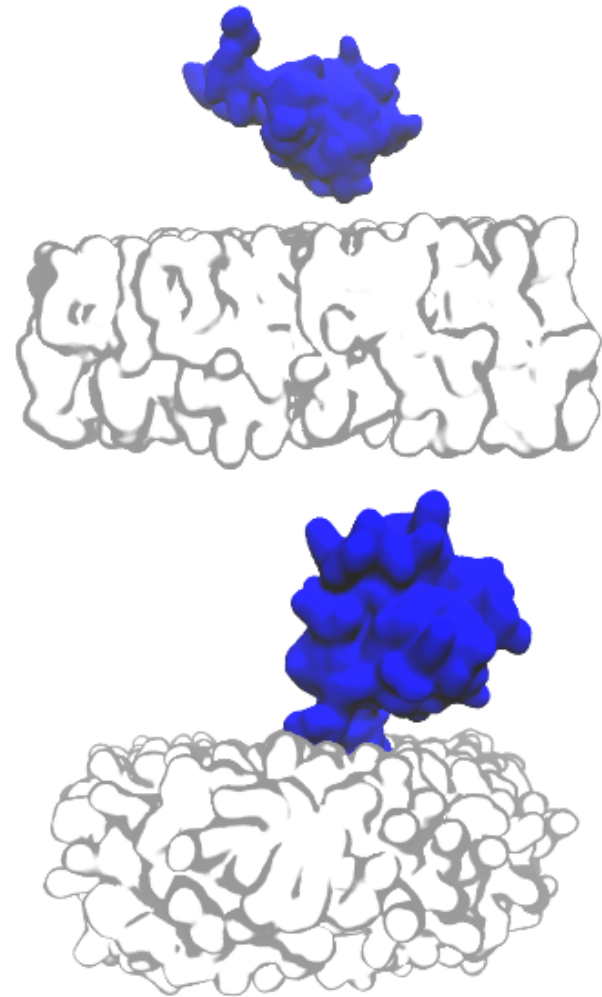
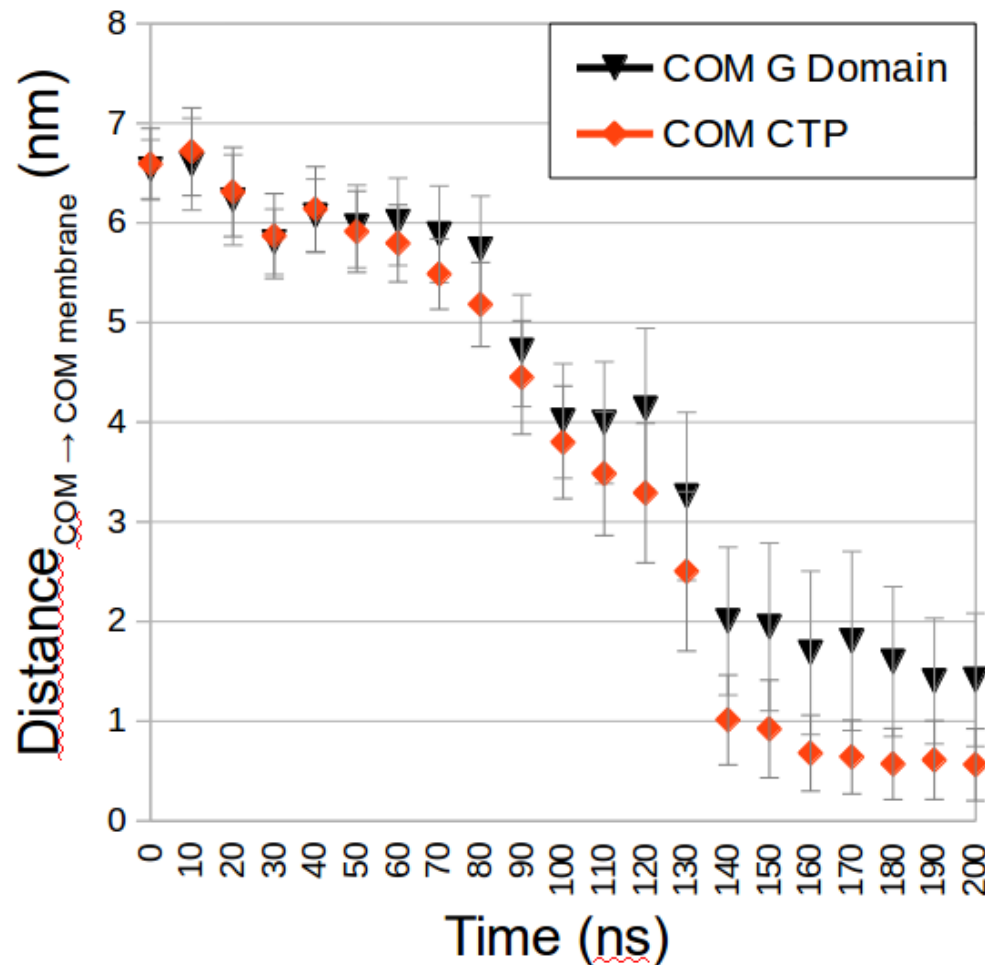
RIT1 deactivation



C terminal peptide-membrane binding



G domain + C terminal peptide membrane binding



A192T

A192T
membrane
binding (7:3)

